PATENT
ATTORNEY DOCKET NO. 50154/002002

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Hubbell et al.	Confirmation No.:	5903
Serial No.:	09/496,231	Art Unit:	1651
Filed:	February 1, 2000	Examiner:	L.B. Lankford
		Customer No.:	21559

Title: BIOMATERIALS FORMED BY NUCLEOPHILIC ADDITION REACTION TO CONJUGATED UNSATURATED GROUPS

DECLARATION UNDER 37 C.F.R. § 1.131 OF PRIOR INVENTION

Under 37 C.F.R. § 1.131, we declare:

1. We are the inventors of the subject matter that is described and claimed in the above-captioned patent application.

2. The enclosed Exhibit is a copy of pages from the laboratory notebook of inventor Alison Pratt, which show that we had reduced to practice the generic invention of the relevant claims prior to August 20, 1998. In particular, these pages show conception of the reaction of multi-cysteine peptides, containing multiple nucleophilic groups, with poly(ethylene glycol) (PEG) acrylates and vinyl-sulfone PEG, containing multiple conjugated unsaturated groups. In addition, these pages report experimental results showing the formation of a gel from the reaction of a polythiol, trimethylolpropane tris(3-mercaptopropionate), with PEG acrylate. The work documented in the Exhibit was performed in Switzerland after January 1, 1996 (but before August 20, 1998).

3. All statements made herein of our own knowledge are true and all statements made on information and belief are believed to be true; and further these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

16 April 07
Date

Jeffrey A. Hubbell

Date

Donald Elbert

Date

Matthias Lutolf

Date

Alison Pratt

Date

Ronald Schoenmakers

Date

Nicola Tirelli

Date

Brent Vernon

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Date	Jeffrey A. Hubbell
April 16, 2007 Date	Donald Elbert
Date	Matthias Lutolf
Date	Alison Pratt
Date	Ronald Schoenmakers
Date	Nicola Tirelli
Date	Brent Vernon

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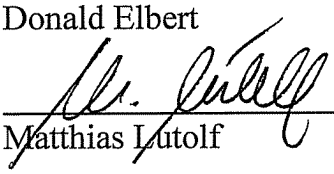
Date	Jeffrey A. Hubbell
Date	Donald Elbert
25.4. 1973 Date	 Matthias Lutolf
Date	Alison Pratt
Date	Ronald Schoenmakers
Date	Nicola Tirelli
Date	Brent Vernon

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Date	Jeffrey A. Hubbell
Date	Donald Elbert
Date	Matthias Lutolf
24. April 2007 Date	Alison Pratt
Date	Ronald Schoenmakers
Date	Nicola Tirelli
Date	Brent Vernon

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Date	Jeffrey A. Hubbell
Date	Donald Elbert
Date	Matthias Lutolf
Date	Alison Pratt
Date	Ronald Schoenmakers
24.04.2007 Date	Nicola Tirelli
Date	Brent Vernon

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Date	Jeffrey A. Hubbell
Date	Donald Elbert
Date	Matthias Lutolf
Date	Alison Pratt
Date	Ronald Schoenmakers
5/4/07 Date	Nicola Tirelli
Date	Brent Vernon

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Date	Jeffrey A. Hubbell
Date	Donald Elbert
Date	Matthias Lutolf
Date	Alison Pratt
Date	Ronald Schoenmakers
Date	Nicola Tirelli
4/17/07 Date	 Brent Vernon

EXHIBIT

(DATES REDACTED)

Gel formation thru w/ polysulfhydryls + acrylates

Goal: to use multi-cysteine peptides w/ enzyme-sensitive sequences to form peg hydrogels in cell-friendly conditions

ie: Cys – plasmin site – Cys – plasmin site – Cys

reacted w/ =⟩peg⟨= instead of

w/ the more expensive Vinyl-sulfone peg

There is enough literature on vinyl-sulfone + S-H reaction, and there are lots of uses for photopolymerization of thiols + enes, however I have not seen much / anything w/ out photopolymerization

Gel formation s/w/ polythiol + peg-DA

Polythiol (tri-thiol):

trimethylolpropane tris(3-mercaptopropionate)

CAS: 33007-83-9 Aldrich

$(HS-CH_2CH_2-COOCH_2)_3C-C_2H_5$

mw = 398.56 d = 1.21 TMP²

acrylate: 8KDA peg (88-107% acrylation, page 107)

conditions:

per Natalie's recommendations from her work w/ vinyl-sulfone peg and cys or cys-containing peptides

the suggested concentrations of 5-10 mM thiol 3x (15-30 mM) vinyl-sulfone

she gels the rxn to go @ pH 7.4, for cysteine, maybe a little faster

Buffer: 50 mM NaBicarb pH 8.4 ($NaHCO_3$)

co-solvent (b/c TMP² not miscible w/water): acetonitrile

acetonitrile / water = 1/1

8KDA: 10% solution 50 mg in 500 µl solvent + cosolvent

peg dissolved, then TMP² added + mixed via vortex

TMP² not perfectly miscible still - appears as suspension of small droplets

let stand for ~30 min: some gelled

" " for ~60 min: all gelled by appearance